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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/843,819	04/30/2001	Tomoko Nakayama	P107424-00027	9941

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EXAMINER	
SAKELARIS, SALLY A	
ART UNIT	PAPER NUMBER
1634	

DATE MAILED: 04/09/2003

Please find below and/or attached an Office communication concerning this application or proceeding.

PTO-90C (Rev. 07-01)

Office Action Summary	Application No.	Applicant(s)
	09/843,819	NAKAYAMA ET AL.
	Examiner	**Art Unit**
	Sally A Sakelaris	1634

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *20 December 2002* .

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-11* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-11* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)

2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)

3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .

4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .

5) ☐ Notice of Informal Patent Application (PTO-152)

6) ☐ Other: .

DETAILED ACTION

This action is in response to Applicant's amendment and response to office action, filed December 20, 2002. Claims 1-3 have been amended, claim 12 has been canceled, and no claims have been added. Claims 1-11 are now pending. Applicant's amendments and arguments have been thoroughly reviewed, but are not persuasive for the reasons that follow. All rejections not reiterated herein are hereby withdrawn. The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action. **This action is Final.**

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

1. Claims 2+3 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

A. Claim 2 is indefinite over the recitation of "nucleic acid inclusion body." The term "nucleic acid inclusion body" is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree and one of ordinary skill in the art would not be reasonably appraised of the scope of the invention. There is no fixed definition in the art for what constitutes a nucleic acid inclusion body. It is unclear, e.g. whether the phrase refers to any body in its entirety, comprising nucleic acids(i.e. an entire animal or plant), or to an isolated sample from just a specific organ/tissue type comprising nucleic acids, or to a single cell comprising nucleic acids, or even just to a purified nucleic acid harvested from one of these three sources prior to amplification...etc. The claims should be amended to clarify what specific sample types are included in the "nucleic acid inclusion body" category.

B. Claim 3 is indefinite over the recitation of "and/or" located between the two steps of adjusting a pH value. It is unclear if both the 25°C step and 70°C step occur in independent reactions, sequentially, singly, multiply, or multiply in sequence. As a result, it is unclear at which point of the reaction and at what frequency pH adjustments occur.

Appropriate correction is required.

-----THE FOLLOWING ARE NEW GROUNDS OF REJECTION-----

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

2. Claims 1, 2, and 4-9, are rejected under U.S.C. 103(a) as being unpatentable over Henke et al. (Nucleic Acids Research, 1997) in view of Watanabe et al.(Neurological Research, 1996).

Henke et al. teach a method for synthesis of nucleic acids to amplify an intended nucleic acid region in which a content of guanine (G) and cytosine (C) is rich, wherein an aliphatic polyhydric alcohol, glycerine is present in an amplification reaction solution. Henke et al. teach this same method for amplifying GC- rich sequences wherein the nucleic acid is a mRNA expressed in the prostatic cancer cell line LNCaP, a living body derived sample, and is added to the amplification reaction solution(Pg. 3957). Furthermore, the reference teaches the aforementioned method for synthesis of nucleic acids wherein the GC content in the GC rich region is 66%(Pg 3957). Additionally, the reference teaches the same method for synthesis of nucleic acids wherein glycerine is contained in a 10% proportion by volume in the amplification reaction solution(Pg. 3957). Lastly, the reference teaches that "since the deletion contains GC-rich sequences(66% GC) the RT-PCR was optimized by both the involvement of DMSO(10%) or 10% glycerine."

Henke et al. do not teach a method for synthesis of nucleic acids to amplify an intended nucleic acid region in which a content of guanine (G) and cytosine (C) is rich, wherein both of the polyhydric alcohol and ammonium sulfate are present in the amplification reaction solution.

However, Watanabe et al. teach a method for synthesis of nucleic acids to amplify an intended nucleic acid region in which a content of guanine (G) and cytosine (C) is rich, wherein an ammonium sulfate is present in an amplification reaction solution. Watanabe et al. teach a reproducible assay of polymerase chain reaction to detect trinucleotide repeat expansion within this GC rich sequence(CAG and CCG flanks). Watanabe et al. further teach the development of a buffer system supplemented with 10mM ammonium sulfate $(NH_4)_2SO_4$ to overcome the difficulty encountered in the amplification of GC-rich sequences such as this(Pgs. 16-18). Watanabe et al. further teach that supplementation with DMSO(10%) and ammonium sulfate $(NH_4)_2SO_4$ was greatly effective to the efficiency of the results(Pg.18).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method Henke et al. so as to have created a method for synthesis of nucleic acids to amplify an intended nucleic acid region in which a content of guanine (G) and cytosine (C) is rich, wherein both a polyhydric alcohol and ammonium sulfate are present in the amplification reaction solution for the expected benefit of an increased efficiency in the amplification of GC-rich sequences. The teachings of both Henke and Watanabe address a similar nature had by both compounds, polyhydric alcohol and ammonium sulfate. Each researcher coupled either polyhydric alcohol or ammonium sulfate to the use of DMSO(10%) in an attempt to amplify GC-rich sequences. This disclosure of each additive's potential for improving the efficiency of PCR involving GC-rich sequences, makes the subsequent combination of such similar, and well known additives obvious to one of ordinary skill in the art.

3. Claims 3 and 11, are rejected under U.S.C. 103(a) as being unpatentable over Henke et al. in view of Watanabe et al.(Neurological Research, 1996) in further view of Bloch (US Patent 5,972,618).

Henke et al. teach a method for synthesis of nucleic acids to amplify an intended nucleic acid region in which a content of guanine (G) and cytosine (C) is rich, wherein an aliphatic polyhydric alcohol, glycerine is present in an amplification reaction solution. Henke et al. teach this same method for amplifying GC- rich sequences wherein the nucleic acid is a mRNA expressed in the prostatic cancer cell line LNCaP, a living body derived sample, and is added to the amplification reaction solution(Pg. 3957). Furthermore, the reference teaches the aforementioned method for synthesis of nucleic acids wherein the GC content in the GC rich region is 66%(Pg 3957). Additionally, the reference teaches the same method for synthesis of nucleic acids wherein glycerine is contained in a 10% proportion by volume in the amplification reaction solution(Pg. 3957). Lastly, the reference teaches that "since the deletion contains GC-rich sequences(66% GC) the RT-PCR was optimized by both the involvement of DMSO(10%) or 10% glycerine."

Henke et al. do not teach a method for synthesis of nucleic acids to amplify an intended nucleic acid region in which a content of guanine (G) and cytosine (C) is rich, wherein both of the polyhydric alcohol and ammonium sulfate are present in the amplification reaction solution.

However, Watanabe et al. teach a method for synthesis of nucleic acids to amplify an intended nucleic acid region in which a content of guanine (G) and cytosine (C) is rich, wherein an ammonium sulfate is present in an amplification reaction solution. Watanabe et. al. further teach the synthesis of nucleic acids wherein a nucleic acid inclusion body in a living body-derived sample, in this case blood(Pg. 17), was added to the amplification reaction solution. The method exemplified by Watanabe amplifies a CAG repeat region of exon 1 of the HD gene. It is a property of the CAG repeat region that it has a GC content of at least 40% and further in the range from 50% to 70%. Watanabe et al. teach a reproducible assay of polymerase chain reaction to detect trinucleotide repeat expansion within this GC rich sequence(CAG and CCG flanks). Watanabe et al. further teach the development of a buffer system supplemented with 10mM ammonium sulfate $(NH_4)_2 SO_4$ to overcome the difficulty encountered in the amplification of GC-rich sequences such as this(Pgs. 16-18). Watanabe et al. further teach that supplementation with DMSO(10%) and ammonium sulfate $(NH_4)_2 SO_4$ was greatly effective to the efficiency of the results(Pg.18).

Watanabe et al. do not teach the method for synthesis of nucleic acids according to claim 1, wherein ammonium sulfate is present at a concentration from 20mM to 100mM in the amplification reaction solution nor does the reference teach increasing the pH at 25°C to 8.4 or higher.

However, Bloch teaches the use of 40mM ammonium sulfate in the PCR amplification reaction solution(Col. 11, line 48 & Col.12, lines 10-13). Block teaches that by using 40mM ammonium sulfate, one could expect that "a pH 9 buffer consisting of ammonium sulfate is especially effective for PCR, instead of the Tris buffer commonly used for PCR.(Col 12, lines 10-13)" An increased pH concentration is well known in the art, to decrease template depurination and thereby enhance PCR. Additionally, Bloch teaches the pH value of the amplification reaction solution at room temperature to be 9.3. As is well known in the prior art, (For example, Yin: US 4,948,724: Col 4: line 59), room temperature is known to be 25°C, therefore obviating the claim to a pH of 8.4 or higher at 25°C.

Therefore, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Henke and Watanabe et al. so as to have increased the ammonium sulfate concentration to 40mM as taught by Bloch in a method to enhance the amplification of an intended nucleic acid region in which a content of guanine (G) and cytosine (C) is rich.

4. Claim 10, is rejected under U.S.C. 103(a) as being unpatentable over Henke et al.(Nucleic Acids Research, 1997) in view of Watanabe et al.(Neurological Research, 1996) in further view of Pomp et al.(Biofeedback, 1991) and in even further view of Fuller(US 5,432,065, 1995).

Henke et al. teach a method for synthesis of nucleic acids to amplify an intended nucleic acid region in which a content of guanine (G) and cytosine (C) is rich, wherein an aliphatic polyhydric alcohol, glycerine is present in an amplification reaction solution. Henke et al. teach this same method for amplifying GC- rich sequences wherein the nucleic acid is a mRNA expressed in the prostatic cancer cell line LNCaP, a living body derived sample, and is added to the amplification reaction solution(Pg. 3957). Furthermore, the reference teaches the aforementioned method for synthesis of nucleic acids wherein the GC content in the GC rich region is 66%(Pg 3957). Additionally, the reference teaches the same method for synthesis of nucleic acids wherein glycerine is contained in a 10% proportion by volume in the amplification reaction solution(Pg. 3957). Lastly, the reference teaches that "since the deletion contains GC-rich sequences(66% GC) the RT-PCR was optimized by both the involvement of DMSO(10%) or 10% glycerine." Generally, the reference teaches the addition of additives such as glycerine, "to ameliorate the amplification of GC-rich DNA sequences," which one skilled in the art recognizes as having previously been unsuccessful or non-optimal under standard reaction conditions.

Henke et al. do not teach a method for synthesis of nucleic acids to amplify an intended nucleic acid region in which a content of guanine (G) and cytosine (C) is rich, wherein the aliphatic polyhydric alcohol is ethylene glycol nor do they teach a method for synthesis of nucleic acids to amplify an intended nucleic acid region in which a content of guanine (G) and cytosine (C) is rich, wherein both of the polyhydric alcohol and ammonium sulfate are present in the amplification reaction solution.

However, Watanabe et al. teach a method for synthesis of nucleic acids to amplify an intended nucleic acid region in which a content of guanine (G) and cytosine (C) is rich, wherein an ammonium sulfate is present in an amplification reaction solution. Watanabe et. al. further teach the synthesis of nucleic acids wherein a nucleic acid inclusion body in a living body-derived sample, in this case blood(Pg. 17), was added to the amplification reaction solution. The method exemplified by Watanabe amplifies a CAG repeat region of exon 1 of the HD gene. It is a property of the CAG repeat region that it has a GC content of at least 40% and further in the range from 50% to 70%. Watanabe et al. teach a reproducible assay of polymerase chain reaction to detect trinucleotide repeat expansion within this GC rich sequence(CAG and CCG flanks). Watanabe et al. further teach the development of a buffer system supplemented with 10mM ammonium sulfate $(NH_4)_2 SO_4$ to overcome the difficulty encountered in the amplification of GC-rich sequences such as this(Pgs. 16-18). Watanabe et al. further teach that supplementation with DMSO(10%) and ammonium sulfate $(NH_4)_2 SO_4$ was greatly effective to the efficiency of the results(Pg.18).

Watanabe and Henke do not teach a method for synthesis of nucleic acids to amplify an intended nucleic acid region in which a content of guanine (G) and cytosine (C) is rich, wherein the aliphatic polyhydric alcohol is ethylene glycol.

However, Pomp et al. teach the use of "ethylene glycol for PCR systems which have previously been unsuccessful or nonoptimal under standard reaction conditions(Pg. 143)", which GC-rich regions would be classified as by one skilled in the art. Further, Fuller exemplifies a method for PCR in which 10 and 50% (v/v) glycerol or ethylene glycol are added to the amplification reaction to destabilize duplex DNA.(5,432,065, Col. 3, lines 10-11). Pomp et al. to expect a benefit as, "it is possible that enhancement of PCR by these[polyethylene glycol] compounds is associated with the general property of organic solvents to destabilize DNA in solution." "Organic solvents cause dehydration in the microenvironment of the DNA, leading to structural perturbation. It is likely that several other similar types of solvents (e.g., 1,2-propanedial, ethylene glycol, methanol) would yield beneficial results for PCR(Pg. 143)." Pomp et al. provide the motivation that, "this could save time and effort in altering other aspects of the system to achieve successful amplification(Pg. 143)." Additionally, the teachings of both Henke and Pomp address a similar nature had by both compounds, glycine and polyhydric alcohol. Each researcher coupled either glycine or polyhydric alcohol to the use of DMSO(10%) to successfully amplify GC-rich sequences. This disclosure of each additive's identical potential for improving the efficiency of PCR involving GC-rich sequences, makes the subsequent combination of such similar, and well known additives obvious to one of ordinary skill in the art.

Therefore, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have used ethylene glycol as the aliphatic polyhydric alcohol in place of glycerine because this would have provided an equally effective means for amplifying regions of high G/C content in addition to a method wherein both of the polyhydric alcohol and ammonium sulfate are present in the amplification reaction solution as both tactics would improve the success of amplifying GC-rich regions.

Response to Arguments

5.

A. Applicant traverses the rejection of claim 12 over Henke in view of Watanabe on the grounds that the motivation to combine the two reference could not be found by a person of ordinary skill in the art. The examiner maintains, however, in the above rejections that the motivation does in fact exist for the combination of these two teachings making obvious applicant's arrival at the claimed amplification reaction solution.

B. Applicant traverses the rejections based on the Henke et al. reference on the grounds that the reference teaches away from adding a polyhydric alcohol such as glycerine in some PCR solutions. The examiner points applicant to page 3957 of the reference in the second paragraph where the "GC-rich sequences(66% GC), the RT-PCR was optimized by both the involvement of 10% DMSO or **10% glycerine**(Fig.1)"(Henke et al.). The reference is clearly teaching the use of glycerine in the attempt to amplify GC-rich sequences. Applicant also respectfully disagrees with the examiner's application of a method for use in amplification of gene fragments instead of for "inclusion bodies" or "living body-derived" samples. Applicant is reminded that these terms used to claim their invention are rejected under the maintained 112 2nd paragraph statutes.

C. Applicant traverses all rejections based on Henke, Watanabe, Barnes, Pomp, Fuller, and Bloch as they fail to teach or suggest the use of both ammonium sulfate and a polyhydric alcohol in an amplification reaction solution as presently amended. The examiner responds by explaining that although each reference individually does not teach the method as claimed, their combination as seen above makes obvious the invention as claimed.

D. Applicant should also further note that with respect to the rejection of claim 10, the motivation for combining the Henke, Watanabe, Pomp, and Fuller references comes from their shared attempt to amplify GC-rich sequences and the new rejections as such are made.

E. With respect to 112 2nd paragraph rejections, applicants state that the previous grounds of rejection have been obviated by the amendments to the claims. However, the rejections are maintained because the amendment and response do not address the limitations in Claim 2 of "nucleic acid inclusion body" or the recitation in claim 3 of "and/or".

6. Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communication from the examiner should be directed to Sally Sakelaris whose telephone number is (703) 306-0284. The examiner can normally be reached on Monday-Friday from 8:00AM-5:00PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion, can be reached on (703)308-1119. The fax number for the Technology Center is (703)305-3014 or (703)305-4242.

Any inquiry of a general nature or relating to the status of this application should be directed to Chantae Dessau whose telephone number is (703)605-1237.

4/08/03

Sally Sakelaris

CARLA J. MYERS
PRIMARY EXAMINER